Histology of the Soft Parts of Astraeid Corals.

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With Plates 7 and 8.

The following account of the histology of the soft parts of the Astraeidae is supplementary to the description given in the Introduction to my paper on 'A Revision of the Recent Colonial Astraeidae possessing Distinct Corallites' (**25**, pp. 1–32). It is based on the study of a large number of polyps belonging to various Astraeid species of the Indo-Pacific and Atlantic regions, particularly of Favia favus (Forsk.), Favia hululensis (Gard.), Coeloria daedalea (Ell. and Sol.), Leptoria gracilis (Dana), Eusmilia aspera (Dana). During a short stay at the Carnegie Marine Biological Station at Tortugas (July 16–August 2, 1915), living colonies of all coral species of that locality were kept under observation, but at that time larvae were extruded only from colonies of Favia fragum (Esp.). These were fixed at different intervals during the free-swimming stage—from eight hours to about ten days—in Flemming's fluid, corrosive acetic solution, and Bouin's fluid, and were subsequently sectioned serially to thicknesses of 4 μ, 6 μ, 8 μ, and 10 μ in order to compare their histology with that of adult colonies. No larvae of any species were obtained during a subsequent visit to the Bermudas (Aug. 20 – Sept. 14). Solid embryos lying in the coelenteric cavities of polyps from a colony of Favia fragum, which Dr. Vaughan forwarded to Professor Gardiner from South Bight, Bahamas, and which have been sectioned, were also studied.

The colonies from the Indo-Pacific region were fixed in saturated solution of corrosive sublimate and in formic aldehyde poured into sea-water; those from the Atlantic region were

narcotized in a partly expanded condition in weak solutions of magnesium sulphate before fixation in formalin. They were then brought up to 75 per cent. alcohol for preservation. The decalcification was done in 2–3 per cent. solutions of nitric acid in 75 per cent. alcohol, some of the colonies with hard coralla taking as long as three months to decalcify, but, as a rule, the histological condition of the soft parts has not been affected to any extent by the process.

Various staining methods were employed, chiefly Haidenhain's iron-haematoxylin followed by eosin, aniline blue, and orange G (Mallory), safranin O, borax-carmine followed by picro-nigrosin and picric aniline blue. Well-preserved polyps were subjected to teasing before and after maceration in the Hertwig's osmic-acetic solution; while at the American Biological stations a similar investigation of fresh coral tissue could not be made to my satisfaction owing to pressure of other work, though it was found that the method of teasing was not so suited as that of serial sectioning to reveal the true histological relationships of lowly differentiated tissues like those of the Madreporaria.

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The soft parts of the Madreporaria consist of an outer and inner protoplasmic sheet and an intermediate supporting lamina. The two former are described in this paper under the widely accepted terms Ectoderm and Endoderm, which had originally been employed by Allman in 1853 to denote the outer and inner layers of the Tubulariadae (**1**, p. 368). But I have refrained from applying any of the suggested names to the middle lamina since (as will be shown in the course of this study), from its nature and formation, this lamina does not appear to be essentially different from the Mesoderm of the Triploblastica. It may also be stated at once that these laminae in Astraeid corals are not discrete layers, as has been the prevalent view, but are of the nature of three strata in a continuous multinucleated sheet.

Ectoderm.

The ectoderm forms the entire outer lining of the soft parts, i.e. of the oral-discs, tentacles, column-walls of polyps (where it is termed the calicoblastic layer), and edge-zones and coenosarc (the ectoderm of their outer wall is a continuation of the oral-disc ectoderm, while that of their inner wall is a continuation of the calicoblastic layer).

In the oral-disc and outer wall of the edge-zone (figs. 1 and 2) the ectoderm has an even free surface which, in sections, is often seen to be covered with mucous secretion and is of more or less uniform thickness. It has a thin free border containing fine vertical striae and is provided with short cilia. Elongated nuclei are aggregated somewhat along the middle of the ectoderm; smaller round ones which are less numerous lie more or less scattered in its inner half. Both mucous and granular vacuoles are present, the former being more abundant than the latter, and a varying number of nematocysts also occur in it. Outwardly diverging tracts are sometimes visible in the protoplasm between the vacuoles. Fibrils are continuous between the ectoderm and middle lamina. At the base of the ectoderm is a finely granular stratum in which a faint network can be discerned which is probably the result of intercrossing of the basal processes of the nuclei of the ectoderm and the fibrils which pass into it from the middle lamina. This stratum has been usually regarded as nervous, but, on renewed examination, no such nerve elements as those described by the Hertwigs in Actinians could be found in it. In the tentacles (fig. 3 and **25**, fig. 44) the ectoderm is greatly thickened at intervals to form the batteries in which nuclei are considerably increased in number, the elongated ones lying in the upper half of the batteries, smaller oval and round nuclei in the lower half. The granular stratum at the base of the tentacular ectoderm is thicker than at the base of the oral-disc ectoderm.

The calicoblastic ectoderm (figs. 10, 13–15) is very thin except where the column-wall processes are being formed, and has a somewhat irregular outline and granular protoplasm, the

granular appearance being more pronounced than elsewhere in the ectoderm. Nuclei are few and are arranged in a single row; they are large, oval or round, rarely elongated, finely granular, and lie tangentially in a single row at comparatively wide intervals, except near the attachments of mesenteries to the corallum, where the calicoblastic layer is usually thickened and nuclei tend to become irregularly distributed without any crowding. Most of the nuclei contain a brilliantly stained spot (perhaps the nucleolus) which is conspicuous under an oil-immersion lens. The calicoblastic layer persists to the base of polyps, though considerably attenuated.

From this account it will be seen that the ectoderm consists of two histologically different regions, viz. the part covering the exposed surface of colonies (i.e. the oral-disc, edge-zone, and coenosarc ectoderm) and the calicoblastic layer, the differentiation being doubtless in accordance with differences in their functions. In the free-swimming larva of Favia fragum, the ectoderm of the column-wall (fig. 4) is histologically similar to that of the oral-disc of the polyp (the calicoblastic layer being non-existent at this stage), and is, in this respect, comparable to the column-wall of Actinians; at the aboral pole the ectoderm is thickened, presumably for future attachment. Tentacles had not appeared in any of the larvae examined.

Endoderm.

The endoderm forms the lining of the coelenteric cavities, i.e. forms the inner lining of the column-wall, oral-disc, edge-zone, and tentacles, the outer lining of the stomodaeum and the double sheet of the mesenteries. It is usually vacuolated and is apparently without cilia, while in Actinians the Hertwigs found a single long cilium or flagellum on each endoderm cell; the vacuoles are often elongated, their longer axes are more or less perpendicular to the width of the mesenteries, the vacuoles being somewhat broader distally. Nuclei are oval or round, smaller and less numerous than those of the oral-disc ectoderm. The endoderm varies in thickness in different parts of the same

polyp and in different species. In the tentacles it is considerably swollen in some species, almost filling their lumina ; in the column-wall it is thin in the stomodaeal region, but becomes highly vacuolated and reticular below this region, where it contains comparatively few nuclei which are arranged in a row near the free surface ; in the stomodaeal wall the endoderm remains uniformly thin. In the mesenteries the endoderm is usually swollen along the pleatal region and behind the filaments. A narrow constriction is present behind the filament, which is deeper in principal than in subsidiary mesenteries (figs. 7 and 8). Numerous round bodies, usually regarded as symbiotic algae, are present in the oral-disc, edge-zone, and tentacular endoderm, i.e. in the exposed regions of colonies ; in some polyps they are so massed as to fill parts of the endoderm. In the mesenteries these algal bodies occur in varying numbers, chiefly in the exocoelic side, but are scarce in the column-wall. Fibrils are continuous between the endoderm and middle lamina, somewhat as between the latter and ectoderm. In all the larval stages examined, the endoderm has attained histological similarity with that of the polyp, but organic débris containing scattered nuclei are still seen in the coelenteric cavity (fig. 4) and are perhaps remnants of the contents of the earlier solid planula stage (**37**, Pl. ii, fig. 4).

The distinguishing characters of the endoderm are its vacuolated condition, presence of algal bodies, the numerical inferiority and somewhat scattered condition of its nuclei. The endoderm, unlike the ectoderm, has a homogeneous appearance, except for its relative swelling in different parts and the varying number of algal bodies present.

Inner Lining of Stomodaeum (figs. 5 and 17).

In the larva, the stomodaeum is said to be formed by invagination of one of its extremities, while it is found that in colony-formation new stomodaea may be formed by invagination or by the union of the broader mesenteries in diverticula (**30**). In the two former cases, the inner lining of the stomodaeum is a continuation of the surface ectoderm, while

in the latter case it is an endodermal formation ; but, whether ectodermal or endodermal in origin, the inner lining possesses histological identity. It is raised into ridges over the attachments of mesenteries (fig. 5) ; these ridges vary in thickness and breadth in different species and frequently possess median grooves as they approach the enterostome. The free border of the inner lining shows the vertical striation better than in the surface ectoderm. It is conspicuously ciliated, the cilia being longer in the median grooves of the ridges and in the sulci between the ridges ; these cilia would function in the ingress and egress of currents of water.[1] Below the striated border is a somewhat finely granular non-nucleated stratum, beneath which is a much thicker region containing massed nuclei of varying length and size (mostly tapering at both ends). Between the nucleated region and the middle lamina is a fibrillar region which is thicker than all other regions and comprises the lower half or two-thirds of the ridge ; it contains a few small, round or oval nuclei, and the fibrils are continuous with the middle lamina. At the base of the inner lining is the fine granular stratum which shades off into the middle lamina. The striated border and the non-nucleated stratum underlying it are of uniform thickness over the entire stomodaeum, while the nucleated and fibrillar regions are considerably thinner in the intermesenterial parts. Vacuoles are usually present in the stomodaeal ridges and are fewer in the intermesenterial region ; many of them contain granules which vary in their density, being quite fine in some vacuoles. The vacuoles extend to the surface of the ridges and, in sections of some polyps, the granules are seen to have actually passed into the lumina of the stomodaea. Nematocysts are sometimes present in the inner lining of the stomodaeum. At the enterostome the inner lining of the stomodaeum becomes continuous with its outer endodermal lining.

Since numerous stomodaea are present in a Madreporarian

[1] Finely powdered carmine, when put into sea-water containing a live colony of Manicina areolata was passed into the stomodaea and subsequently ejected.

colony, the ectoderm of the free surface is continuous with the stomodaeal lining at frequent intervals. The inner lining of the stomodaeum of the larva is not raised into ridges at the mesenterial attachments, hence both grooves and sulci are absent ; its nuclei are not so crowded together nor so slender as in the polyp, and are arranged along the middle of the layer whose protoplasm is conspicuously granular and opaque above the nuclei, while below them it is vacuolated and translucent ; the condition of the stomodaeal lining of the larva is on the whole intermediate between that of the oral-disc ectoderm and the stomodaeal ridges of the polyp.

Mesenterial Filaments (figs. 7, 8, and 18).

The epithelium of mesenterial filaments has essentially the same structure as the inner lining of the stomodaeum, the median lobe being similar to the stomodaeal ridge and the ventro-lateral tracts to the parts between the ridges. The median grooves of most of the stomodaeal ridges are continued to some distance along the middle of the straight regions of their corresponding mesenterial filaments, the cilia in the grooves being longer than those over the rest of the filaments. A transverse section through a principal filament just below the stomodaeum, as shown in fig. 6, bears striking resemblance to Ashworth's figure of a transverse section through a dorsal mesenterial filament of Xenia Hicksoni (**3**, fig. 19). Granular vacuoles are frequently present in the straight region of the filaments. Nematocysts are few in the straight region, numerous in the convoluted parts, where they often become massed together. Each of the ventro-lateral tracts of a filament is organically continuous with the mesenterial endoderm on its side. Filament-epithelium is present on subsidiary mesenteries (except on the very narrow ones) along the greater part of their length, but is rudimentary in their upper half or one-third, where it contains a few aggregated nuclei or is sometimes entirely absent. Subsidiary filaments are smaller in transverse section than principal filaments. Mesenterial filaments of the free-swimming larva (whether of principal or

subsidiary mesenteries) are similar to the inner lining of its stomodaeum, i.e. nuclei in them are not so closely aggregated nor so slender as in filaments of polyps.

It is obvious that in a subsidiary mesentery of a polyp the filament is formed by modification of the endoderm of the mesentery along its free margin, attaining histological similarity with the inner lining of the stomodaeum and with filaments of principal mesenteries. Stages in this modification are seen in subsidiary mesenteries of varying width. In larvae of Favia fragum, also, filament-epithelium is present along the margins of some subsidiary mesenteries which is undoubtedly formed by modification of the marginal endoderm of those mesenteries. I have previously described the presence of filament-epithelium on the mesenteries of an extra-tentacular bud of Favia hululensis (Gard.) (**27**).

In some species, Favia fragum, Mercilina ampliata, Hyderophora maldivensis, Isophyllia dipsacea, there are regions in the convolutions of mesenteries in which the filament-epithelium is considerably vacuolated and swollen, in which nematocysts i or ii are closely arranged. In dumb-bell-shaped transverse sections of these, the filament-epithelium at each end resembles the intervening endoderm. In other words, histologically identical epithelia are found in stomodaea and mesenteries, whether the inner linings of the former and the filaments of the latter are ectodermal or endodermal in origin. But it is to be noted that algae are absent from the inner lining of the stomodaeum and mesenterial filaments.

The Supporting Middle Lamina.

The middle lamina is found everywhere between the ectoderm and endoderm and forms the median core of mesenteries. Though Bourne could find no trace of structure in the middle lamina of Fungia, he remarked that the use of proper reagents might possibly have disclosed a fibrillar structure (**5**, p. 310). As a result of making careful microscopical preparations this lamina is now found to consist of (1) a homogeneous matrix or clear cementing substance containing (2) fine fibres

and (3) nuclei (figs. 1, 11, and 12). The fibres are of two kinds : those which have a wavy appearance and run in various directions but have chiefly a longitudinal and transverse disposition—such fibres appear to be unbranched and are closely cemented together to form the substance of the lamina ; branching fibres which form a loose plexus in the lamina—these are brought to view by carefully staining sections of not more than 6 μ thickness. The apparently homogeneous appearance of the middle lamina is due to the thinness and close cementing of the fibres. Nuclei are comparatively few and lie scattered in the lamina ; they become evident in tangential or oblique sections through the thicker regions. Each nucleus is oval in shape, containing a conspicuous spot (the nucleolus), and lies in thin finely granular protoplasm from which irregular processes usually radiate into the substance of the lamina ; not infrequently a narrow clear space can be detected around the protoplasm. In several West Indian species of coral Duerden noted the presence of ' migrant connective-tissue cells, such as occur in the larger Actinians ' (**9,** p. 22).

The middle lamina is thickened in the mesenteries and is raised on one side into longitudinal pleats whose breadth and thickness vary in the different species (fig. 9). In the stomodaeal region the pleats extend over part of the width of mesenteries to a varying distance from their column-wall attachments, while below the stomodaeum they cover almost the entire width of mesenteries. The lamina is usually considerably thickened where mesenteries join stomodaea and column-walls. While at the stomodaeal attachment the thickening is restricted to the ridge, at the column-wall insertion the thickening usually spreads a short distance into the adjacent middle lamina, these lateral thickenings appearing, in transverse section, like two arms. The middle lamina is thickened to a less extent in the tentacles and oral-disc ; in the former, outer longitudinal pleats are present which are less conspicuous than those of mesenteries. Processes arise from the middle lamina over the entire extent of the column-wall to attach the soft parts to the corallum, and are more numerous

and larger at the insertions of mesenteries. These processes are composed of fibres and cementing substance, and are the homologues, in the column-wall, of the pleats in mesenteries and tentacles.

The superficial longitudinal fibres in the pleats of mesenteries and tentacles are specially thickened. These specialized fibres, which vary in thickness, appear to be composed of fibrils, but had usually been supposed to be similar to the muscular elements described by the Hertwigs in Actinians, Faurot in 1895 being the first to doubt their muscular nature. In teased preparations and in sections, nuclei are not found in these fibres nor is there any morphological or physiological evidence for regarding them as muscular. Specialized fibres are present on the exocoelic side of mesenteries (but not so thick nor so close together as on the entocoelic side), although pleats are absent from that side or only a few feebly developed ones are present near the stomodaeal attachment. The striae in the processes of attachment appear to be specialized fibres which have a radial disposition.

In preparations of mesenteries with the endodermal lining scraped off, the specialized longitudinal fibres of the middle lamina could be seen running along its entire length. When parts of the living tissue of Isophyllia were isolated from expanded colonies, teased in sea-water, and stained in methylene blue, the middle lamina took a purple or violet tinge and its fibrous texture became quite apparent. The fibrous condition could also be unmistakably seen in properly preserved tissue which had been teased after maceration and removal of the protoplasmic sheets, as well as in such tissue cut to 4 μ and 6 μ thicknesses. The branching fibres were best seen by staining in safranin O and picro-nigrosin. The specialized fibres of the middle lamina, whether in the pleats or in the processes of attachment, were similarly coloured with different stains, e.g. dark in iron haematoxylin, purple in aniline blue and orange G, slaty blue in borax-carmine followed by picro-nigrosin, such results suggesting identity of texture of both sets of fibres. The absence of muscular fibres in the

soft parts of the Astraeidae would also explain the absence of a nervous system—central or peripheral.

In the larval stage the middle lamina is present everywhere and is fibrous, though thinner than in the polyp. Pleats are hardly recognizable in mesenteries, but specialized fibres are present. H. V. Wilson found that in the development of Manicina areolata the middle lamina appears in the solid planula stage (**37**, fig. 5). This observation is corroborated by my study of the solid embryonic stages occurring in the coelenteric cavities of polyps of Favia fragum.

The middle lamina of Actinians as seen from a study of serial sections of young polyps of Sagartia bellis, Metridium senilis, and Corynactis viridis (which in alcohol measured 2 mm. × 1 mm., 12 mm. × 3 mm., and 4 mm. × 3·5 mm.), obtained from Plymouth, is in essential points similar to that of Astraeid corals. In the former two species, the middle lamina has a swollen somewhat loosely spongy core containing many nuclei which is bounded by closely arranged unbranched fibres; the plexus of the spongy core consists of branching fibres which are more abundant than in coral polyps (fig. 16). In Corynactis viridis the meshwork is closer, approaching the condition in the Astraeidae. The principal difference is that, in the column-wall of Actinians, the middle lamina is considerably thickened. The fibrous condition of the middle lamina of various Zoantharia had been previously observed by Kölliker, Schneider, and Rötteken, von Heider, Jourdan, the Hertwigs, and Faurot.[1] As early as 1875, Allman remarked that the 'hyaline lamella' (= middle lamina) of Myriothela consisted of 'two layers—internally a perfectly transparent, thin, structureless membrane, and externally a layer of fibrillae, which adheres closely to the structureless membrane' (**2**, p. 554, fig. 6).

The histology of the middle lamina of the Madreporaria resembles that of mammalian connective tissue, the massed

[1] Hickson in 1883 described the middle lamina of Tubipora as consisting of a 'homogeneous matrix' in which might be found 'cells and fibres' (**17**, p. 11).

wavy unbranched fibres, the network of branching fibres, and the nuclei with the granular protoplasm in which they lie, are comparable respectively to the white fibres, branching fibres, and the so-called connective-tissue 'corpuscles'; the various elements lie in a clear matrix in both cases. It is probable that the branching fibres in the middle lamina of coral colonies are elastic like those of connective tissue.

The possibility has not been excluded that the fibrous strands of the 'epithelio-muscular' or 'myo-epithelial' cells, so commonly figured in connexion with the histology of Coelenterates, might only be fibres of the middle lamina torn apart with the adjacent parts of the ectoderm and endoderm in the process of teasing. Such results are to be expected; the ectoderm, middle lamina, and endoderm are organically continuous. (In teasing, protoplasmic parts are sometimes dissociated from the fibres of the middle lamina, as in Hickson's figures 28 *b–e* of Alcyonium digitatum.) It is not improbable that, as in the Astraeidae, these strands may be of the nature of connective-tissue fibres, for, in figured examples of epithelio-muscular cells, the nuclei, unlike their position in plain muscular fibres of mammals, lie in the protoplasm extrinsic to the strands (**16,** Pl. vi; **18,** Pl. xxxix; **3,** Pl. xxvi).

Bourne, H. V. Wilson, Duerden, and others regarded the middle lamina of the Madreporaria as a secretion of one or both of the protoplasmic layers and 'not formed by the direct metamorphosis of the ends of ectoderm or endoderm cells' (**37,** p. 198). The appearances in my various preparations, however, suggest that the middle lamina is formed by modification of part of the protoplasm of the ecto-endoderm into cementing substance and fibres, all or some of the nuclei in the modified part of the protoplasm becoming the nuclei of the lamina. The formation of the middle lamina is well seen in the case of the processes of attachment which are formed in the calicoblastic layer, stages in their development being abundantly present in my preparations (figs. 13–15). Where such a process is to be formed, the calicoblastic layer is raised

into a short, somewhat irregular eminence which may or may not contain a nucleus. Subsequently, the protoplasm of this projection becomes modified from its periphery inwards to the middle lamina of the column-wall and specialized fibres appear in it. At the attachments of mesenteries to the corallum, the calicoblastic projections are usually larger and each of them often contains more than one nucleus ; when their protoplasm has been modified, the attaching structures become connected with the middle lamina usually by means of narrow necks, while elsewhere they are smaller and arise directly from the middle lamina. In my preparations there is no indication that these processes are at first formed in cellular elements or 'desmocytes' which become subsequently connected with the middle lamina by the modification of neighbouring 'cells' of the calicoblastic layer, as Bourne described (p. 329), but they are the result of a continuous change in the multinucleated calicoblastic layer, the transformation of the protoplasm beginning from its periphery and gradually extending inwards to join the middle lamina. The processes are the parts that project beyond the outer margin of the calicoblastic layer and in which the specialized fibres lie. These fibres are pronounced towards the periphery of the processes, gradually becoming fainter as they reach the middle lamina, and probably they merge into the fibrils of the latter.[1]

Part of the middle lamina is formed entirely in the ectoderm, viz. the processes of attachment in the calicoblastic

[1] According to Bourne in *Caryophyllia Smithii*, 'where a desmocyte is about to be formed, one, two, or three nuclei become surrounded with a mass of darker, finely granular protoplasm. The next phase is the appearance of a band-shaped or ovoid body in the centre of the granular protoplasm which already shows faint signs of striation . . . usually one nucleus remains in close association with this body; the others (if more than one combine to form the granular protoplasmic mass) appear to be concerned in the formation of the mesogladal process which will join the desmocyte to the mesogladal lamina. The striations next become more defined, and the desmocyte, which was at first separate from the mesoglada, becomes attached to it by a process developed, as it seems, at the expense of neighbouring cells' (pp. 528–9). (A desmocyte containing more than one nucleus he regarded as a cell.)

layer, part of it arises in the endoderm, viz. the median core of mesenteries, and the remainder is contributed to by both the ectoderm and endoderm, viz. the middle lamina of the column-wall, oral-disc, edge-zone, and stomodaeum. While Bourne in 1899 held that the processes of attachment, which were essentially similar to and became part of the middle lamina, were formed by modification of elements in the calicoblastic layer or ' desmocytes ', i.e. were intra-protoplasmic formations, he had in a previous paper (**5**) inferred that the middle lamina itself was a secretion of ectoderm and endoderm, i.e. was an extra-protoplasmic product.

The middle lamina appears to be essentially a supporting stratum, i.e. has the function of connective tissue of Vertebrates and, like the latter, has a fibrous texture. It is best developed in mesenteries, since they support the oral-disc with the tentacles and keep the stomodaeum in position, the longitudinal pleats giving additional strength to the mesenteries. Owing to the presence of a calcareous skeleton to support the column-wall, the middle lamina in the Madreporaria is very thin, whilst in Actinians the absence of such a skeleton has necessitated a considerable thickening of the middle lamina in the column-wall (being best developed in this region), which, when the column-wall is folded longitudinally as in Metridium senilis, is swollen into longitudinal ridges within the folds or rugae (fig. 16). The column-wall processes are analogous to tendinous structures in Vertebrates, since, doubtless, they attach the soft parts to the corallum. This function would account for their sucker-shape, usually concave attaching surface, and comparatively small size, combined with their numerical abundance ; the specialized fibres in them presumably impart additional toughness to these processes.

Since the middle lamina has a spongy texture, the infilling of its meshes with fluid would help in the distention of polyps, which is, however, mainly effected by the ingress of sea-water into the polyp cavities, while the general contractility of the middle lamina would help in the retraction of polyps.

General Considerations.

In the tissues of the Astraeidae, cell-limits cannot be discerned, the nuclei lying immersed in the general protoplasm. This is particularly the case in the surface ectoderm, inner lining of stomodaeum, and in mesenterial filament. While in the endoderm, nuclei tend to lie between vacuoles, in the middle lamina nuclei are few and the protoplasmic areas containing them are not definitely circumscribed but appear to be organically connected together by means of their radiating strands. Sections of 4 μ and 6 μ thicknesses treated with silver nitrate failed to show any cell-limits, nor is a cellular structure seen in sections cut in gelatin with Aschoff's CO_2 freezing microtome, nor again in celloidin sections of polyps. Duerden observed that in Siderentsea radians the endoderm of the wall of the polyp lining the uppermost parts of the skeleton is 'a syncitium showing no signs of cellular divisions', and that the calicoblastic layer 'in the growing areas of the skeleton shows no evidence of cell limitations' (**9**, pp. 30, 31). Gardiner, too, could not find definite cell outlines in Coenopsammia and Flabellum (**13** and **14**). Such outlines, so frequently represented in figures of the ectoderm and endoderm of the Anthozoa, are doubtless conventional and arbitrary.

The products of teasing of the tissues, whether before or after maceration, cannot be regarded as separated units of structure or 'cells', but are really bits of protoplasm inevitably torn apart with the nuclei in the mechanical process of teasing. Hence, it is generally found that such pieces of protoplasm possess neither regular nor uniform contour and are sometimes torn apart with fibres of the middle lamina. If an appearance of cellular strands is noticeable in some preserved tissues, it is due to the shrinkage of protoplasm around the nuclei, which probably act as centres of force. H. V. Wilson regarded the endodermal mass of the solid planula stage of Manicina areolata as a 'plasmodium which was subsequently broken up into cells' (**37**, p. 200): he regarded the earlier blastosphore as composed of cells, although their inner ends were 'not

distinctly marked off from the solid endoderm' (p. 197). In many of Bourne's figures of the soft parts of the Anthozoa definite cell boundaries are not visible, although such limits have been presupposed in the descriptions. Indeed, in Caryophyllia, Euphyllia, Madrepora, and 'several other' corals Bourne could not find any cell outlines in the calicoblastic layer (**7**, p. 532), the latter being an irregular multinucleated sheet of protoplasm. When more than one nucleus was present in a mass of protoplasm, it was assumed to be a coenocyte formed by the fusion of uninucleated cells; for example, in referring to scleroblasts or spicule-forming cells of Alcyonaria, Bourne remarked that they were 'often coenocytes containing two, three, or more nuclei' (p. 509). It would appear to be more likely that the scleroblastic tissue was of the nature of a syncytium in which spicular bodies formed.

The mucous and granular vacuoles in the outer lamina of the Madreporaria have also been regarded as cells, but nuclei are not definitely related to them, some of them having more than one nucleus while others show none at all. The only cellular elements in the soft parts of the Astraeidae are nematocysts, algal bodies, and the reproductive elements; these are all characterized by their definite and uniform outline. Nematocysts are secondary formations in the ectoderm for special purposes. Algal bodies are restricted to the endoderm, but little is known of their life-history; it is doubtful if they are symbiotic organisms, as is generally supposed, since they are found in newly hatched larvae and even in earlier embryonic stages (**37**, Pl. ii, fig. 4). Ova and spermatozoa lie in spaces in the middle lamina (**25**, figs. 9, 10, and 49).

The laminae of Astraeid corals are therefore to be regarded as syncytial, and since, as has been seen, there is organic continuity between them, i.e. the ectoderm is everywhere directly continuous with the middle lamina and the latter with the endoderm, and, further, the ectoderm passes into the endoderm by way of the inner linings of the stomodaeum, the tissues form one nucleated continuum which has undergone

partial differentiation into three strata. Towards the base of the column-wall, the middle lamina is absent in places, the calicoblastic layer and the endoderm merging into each other. In such places the appearance is that of one sheet of nucleated protoplasm with an outer granular stratum containing large oval nuclei tangentially placed at intervals, which represents the calicoblastic layer, and an inner stratum whose nuclei are smaller but more numerous and placed vertically, which represents the endoderm (fig. 10).

Since the middle lamina of the Astraeidae is nucleated, formed early in development, and is of the nature of connective tissue, it is comparable to the mesoblast and mesoderm of other animals.[1] Bourne in 1887 restricted these terms to denote the intermediate layer of the triploblastica (which he apparently identified with the coelomata), on the view that the middle lamina of Coelenterates was neither embryonic nor ' cellular ', to which he gave a new name, mesoglaea (**5**, p. 311). This nomenclature was subsequently accepted by most authors—Haddon, van Beneden, Hickson, Ashworth, McMurrich, Duerden—who regarded the nuclei occurring in the middle lamina of Coelenterates as belonging to cells which secondarily migrated into the gelatinous secretum from one or both of the protoplasmic laminae—a view to which my studies on the Madreporaria lend no support. Moseley, von Heider, O. and R. Hertwig, and other earlier zoologists had described the intermediate layer of the Anthozoa under the term mesoderm. This prior usage was resumed in 1895 by Faurot, who, from his comparative study of many Actinian species, disagreed with Bourne in regard to its supposed extra-protoplasmic formation and structureless consistency and the need for a new terminology. It is also clear from the embryological

[1] Bourne states that ' by mesoblast is meant a layer of undifferentiated cells, developed in the embryo before the differentiation of other organs or tissues from either one or the other or both of the primary germ-layers, the epiblast and hypoblast. By mesoderm and its adjective mesodermic are meant all such tissues in the adult as are clearly derived from the mesoblast ' (**5**, p. 314).

studies of Jourdan on Actinia equina and Balanophyllia regia, of E. B. Wilson on Renilla, and of H. V. Wilson on Manicina areolata, that the middle lamina appears early in development—in the solid planula stage. I have found this to be the case in the solid embryos of Favia fragum. Jourdan distinguishes between a 'membrana propria' and a granular mass; while the origin of the former was uncertain, the latter was said to be formed by the severance and fusion of the inner ends of the ectoderm cells of the body-wall, which subsequently become fibrous.[1] E. B. Wilson, who made a more or less similar distinction, also found that the middle lamina of the body-wall was formed by the separation and fusion of the swollen inner ends of ectoderm cells, though he somewhat arbitrarily termed the process 'a peculiar form of cuticular secretion' (**36**, p. 759). Bourne's account of the formation of the middle lamina in Heliopora (**6**) is not different from those of Jourdan and E. B. Wilson.

Although a discussion of the highly controversial subject of the history and homology of the germ-layers of the Metazoa does not lie within the scope of this paper, it will be seen from the foregoing account that there was not adequate reason for withholding the application of the term mesoderm to the middle lamina of the Anthozoa.

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[1] The mode of formation of the middle lamina of certain Alcyonarians described by Kowalevsky and Marion (**23**) is essentially similar to that of Jourdan, but in their subsequent discussion they, however, came to the conclusion that the middle lamina of Coelenterates was not homologous with the mesoderm of Coelomates.

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EXPLANATION OF PLATES 7 AND 8.

Lettering employed.

alg., algal bodies. *c.l.*, calicoblastic layer. *ect.*, ectoderm. *end.*, endoderm. *gr.st.*, granular stratum at base of ectoderm. *gr.v.*, granular vacuole. *lg.f.*, longitudinal fibres of middle lamina. *m.f.*, mesenterial filament. *m.l.*, middle lamina. *muc.v.*, mucous vacuole. n_1, type I nematocyst. n_2, type II nematocyst.

Fig. 1.—Coeloria daedalea (Ell. and Sol.). Part of somewhat oblique section through oral-disc and mesentery.

Fig. 2.—Eusmilia aspera. Part of vertical section through edge-zone. The endoderm is crowded with algal bodies.

Fig. 3.—Coeloria daedalea (Ell. and Sol.). Part of transverse

section through a tentacle showing a sub-terminal battery. Since the section was cut somewhat obliquely, the middle lamina and its specialized longitudinal fibres (*lg.f.*) appear thicker.

Fig. 4.—Larva of Favia fragum (Esp.) ten hours after extrusion. Part of transverse section through column-wall. Note fibrous condition of middle lamina and protoplasmic remains in coelenteric cavity.

Fig. 5.—Coeloria daedalea (Ell. and Sol.). Part of transverse section (slightly oblique) through stomodaeum, showing a ridge and adjacent intermesenterial areas.

Fig. 6.—Ibid. Part of transverse section through a principal mesentery just below stomodaeal region, showing continuation of median groove on filament.

Fig. 7.—Ibid. Part of transverse section through a principal mesentery below stomodaeal region, showing straight region of filament and the two endodermal lobes.

Fig. 8.—Ibid. Part of transverse section through a subsidiary mesentery, showing straight region of filament and the two endodermal lobes.

Fig. 9.—Ibid. Part of transverse section through pleatal region of a principal mesentery in stomodaeal region.

Fig. 10.—Favia hululensis (Gard.). Part of transverse section through column-wall at base of polyp. The middle lamina is thin and somewhat discontinuous at this level.

Fig. 11.—Coeloria daedalea (Ell. and Sol.). Longitudinal fibres of the middle lamina of a mesentery after maceration in osmic-acetic solution and staining in borax-carmine and picro-nigrosin.

Fig. 12.—Ibid. Part of tangential section (6 μ thick) through a mesentery, showing the network of branching fibres of the middle lamina. Note the mass of unbranched fibres and nuclei in the middle lamina.

Figs. 13–15.—Showing stages in the formation of column-wall processes in the calicoblastic layer.

Fig. 13.—Leptoria gracilis. Part of transverse section through intermesenterial region of column-wall at level of stomodaeum.

Fig. 14.—Ibid. Part of transverse section through column-wall at attachment of a mesentery in stomodaeal region.

Fig. 15.—Coeloria daedalea (Ell. and Sol.). Part of transverse section through column-wall at attachment of a mesentery in stomodaeal region.

Fig. 16.—Metridium senilis. Part of transverse section through column-wall, showing a ridge. In the middle lamina note (1) the swollen loosely spongy core which is less open towards the ectoderm and endoderm, the network itself consisting of branching fibres; (2) circularly arranged unbranched fibres bounding the spongy core, the fibres being massed against the endoderm; (3) nuclei in the spongy core; a thin granular protoplasmic area can be seen around most of them. The points marked

in the meshwork are probably transverse sections of longitudinal fibres, being more numerous where the meshwork is less open.

Fig. 17.—Larva of Favia fragum (Esp.) ten hours after extrusion. Transverse section through stomodaeum. Four mesenteries have joined stomodaeum ; their stomodaeal attachments are shown in figure.

Fig. 18.—Larva of Favia fragum (Esp.) ten hours after extrusion. Part of transverse section through a primary mesentery, showing filament.